2:4-DINITRO-PHENYL-HYDRAZINE

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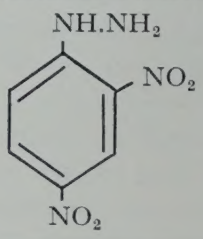
CONTENTS

2:4-DINITRO-PHENYL-HYDRAZINE

In 1920 Mathewson[1] estimated small quantities of water-soluble carbonyl compounds by forming the 2:4-dinitro-phenyl-hydrazones. In 1926 Brady and Elsmie[2] suggested 2:4-dinitro-phenyl-hydrazine as a reagent for identifying aldehydes and ketones, the dinitro-phenyl-hydrazones formed on reaction with compounds containing a carbonyl group being usually crystalline, relatively insoluble products; the reaction has consequently been applied to both qualitative and quantitative analysis of organic carbonyl compounds. Today 2:4-dinitro-phenyl-hydrazine is one of the standard reagents in most laboratories, where it is frequently used instead of phenyl-hydrazine for characterising carbonyl compounds, a dinitro-phenyl-hydrazone often being crystalline where the corresponding unsubstituted phenyl-hydrazone is a low-melting derivative requiring further recrystallising. The reagent appears in the U.S. Pharmacopoeia and in the British Pharmacopoeia, where it is employed in the assay of camphor. In 1935 Strain[3] separated β-ionone and camphor, geronic acid and laevulinic acid by passing a mixture of the 2:4-dinitro-phenyl-hydrazones through a talc chromatographic column; a similar technique has been successfully employed in the biochemical and steroid fields, and is now widely used in separations of carbonyl compounds.

SOME PROPERTIES & REACTIONS OF 2:4-DINITRO-PHENYL-HYDRAZINE

Formula: 

Molecular weight 198.14

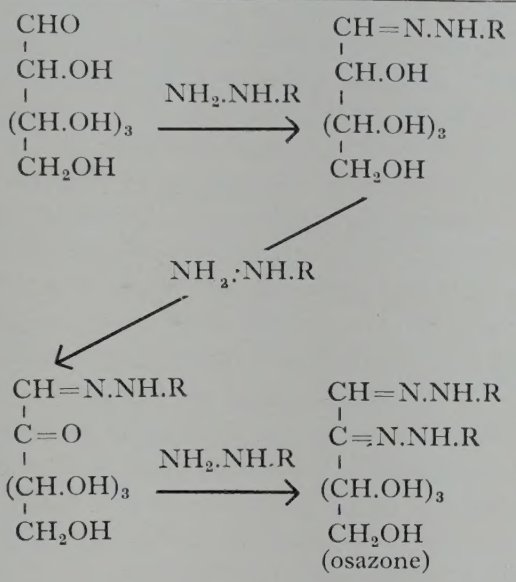
2:4-Dinitro-phenyl-hydrazine is a crystalline powder melting at about 196-200°C with decomposition. It is insoluble in water; moderately soluble in hot ethanol; sparingly soluble in diethyl ether, chloroform, benzene and carbon disulphide; soluble in aniline and in hot ethyl acetate; and soluble in moderately weak mineral acids.

It readily forms hydrazones with a variety of aliphatic, cyclic, and aromatic carbonyl compounds:

$$\begin{matrix}R_1\\R_2\end{matrix}\!\!>\!CO + NH_2.NH.C_6H_3(NO_2)_2 \rightarrow \begin{matrix}R_1\\R_2\end{matrix}\!\!>\!C = N.NH.C_6H_3(NO_2)_2 + H_2O$$

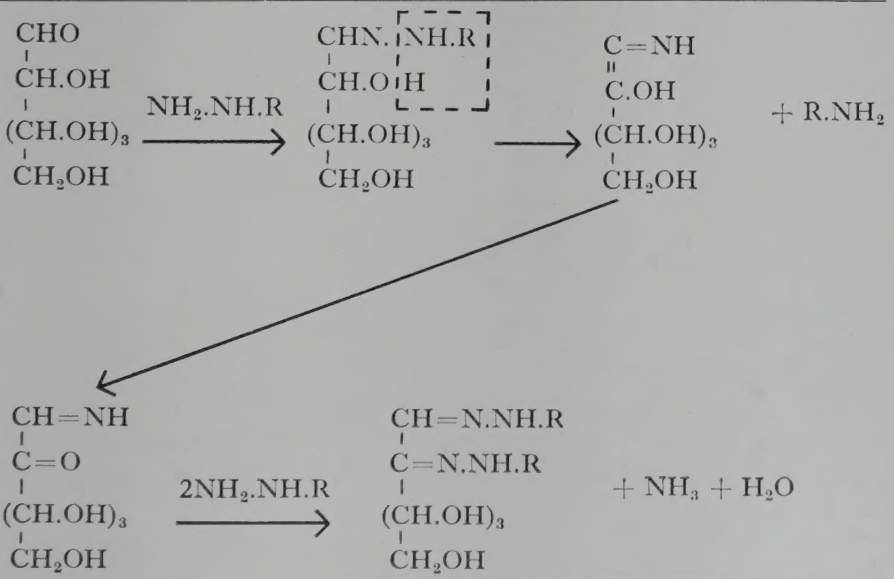
Derivatives of Sugars

Sugars react with the dinitro-phenyl-hydrazine to form substituted osazones, usually crystalline derivatives. (The name 'osazone' is a combination of '-ose' from the sugar and '-zone' from the hydrazone). According to one theory the dinitro-phenyl-hydrazone is first formed. The $>$CH.OH adjacent to the dinitro-phenyl-hydrazine group is oxidised to $>$CO by an excess of the dinitro-phenyl-hydrazine, and reacts with another equivalent of the reagent:



[R = —$C_6H_3(NO_2)_2$]

Another explanation[4] is that the reaction proceeds as follows:



More recent studies[5,6] of osazone formation using C^{14}-labelled phenyl-hydrazine suggest that a mol. of R.NH.NH_2 adds to the enolic form of the phenyl-hydrazone as postulated by Fieser and Fieser[7].

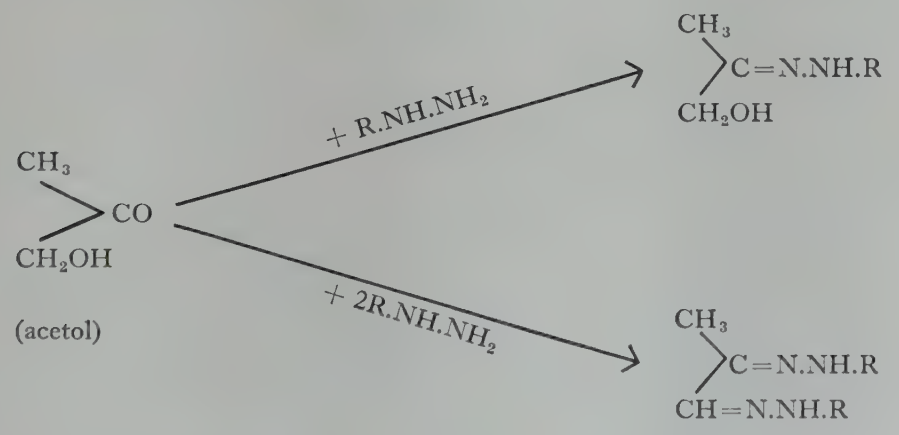
Glaser and Zuckerman[8] prepared and isolated the osazones and intermediate 2:4-dinitro-phenyl-hydrazones of α-glucoheptose and of glucose; because of the insolubility of glucose 2:4-dinitro-phenyl-hydrazone in ethanol, and the fact that it is easily cleaved with formaldehyde, the derivative was recommended for separating glucose from other sugars.
The same preparative conditions were employed by Lloyd and Doherty[9], who prepared 2:4-dinitro-phenyl-hydrazones of some hexoses and pentoses. The method was as follows:
A sample of the sugar, 0.02 mole, was dissolved in 5 ml of water, and a suspension of 0.02 mole of 2:4-dinitro-phenyl-hydrazine in 100 ml ethanol absolute was added. The mixture was refluxed for 12 hours and the resulting clear yellow to red solution was evaporated to dryness *in vacuo;* the residue was extracted with 50 ml hot ethyl acetate to remove small quantities of red oxidation product. The dinitro-phenyl-hydrazones were recrystallised from 80-95% ethanol. (The galactose derivative was highly insoluble, and was purified by several extractions with hot ethyl acetate).
The melting points of some of the 2:4-dinitro-phenyl-hydrazones are shown below:

SUGAR	MELTING POINT °C	REFERENCE
α-Glucoheptose	180–1	8
	(osazone 231–2)	8
D-Glucose	118–122	8
	122–4	9
	(osazone 256–7)	8
D-Mannose	180–1	9
D-Galactose	178–9	9
D-Arabinose	181–2	9
D-Ribose	165–6	9
D-Xylose	162–3	9
D-Lyxose	169–170	9

Derivatives of Alcohols and Ketols

Braude and Forbes[10] have reported yields of from 5 to 25% of the 2:4-dinitro-phenyl-hydrazones of aldehydes and ketones formed from primary and secondary alcohols containing two or more ethylenic or aromatic substituents conjugated with the carbinol group. The aldehydes and ketones are considered to be the products of oxidation of the alcohols by 2:4-dinitro-phenyl-hydrazine:

In a detailed study of the reactions of certain simple ketols with 2:4-dinitro-phenyl-hydrazine Reich and Samuels[11] reported that acetol (hydroxy-acetone) and its acetate yield the dinitro-phenyl-hydrazone or pyruvaldehyde bis-2:4-dinitro-hydrazone, according to conditions.



Similarly dihydroxy-acetone and DL-glyceraldehyde gave the mono- and di-(2:4-dinitro-phenyl-hydrazones); benzyl-carbinol yielded a 2:4-dinitro-phenyl-hydrazone together with a small amount of phenyl-glyoxal di-(2:4-dinitro-phenyl-hydrazone).

When preparing 2:4-dinitro-phenyl-hydrazine derivatives of highly oxygenated carbonyl compounds Wolfrom and Arsenault[12] found that the reaction of α-hydroxy-carbonyl compounds with a super-saturated solution of the reagent in 2N hydrochloric acid, or a solution in boiling 90% ethanol, gave hydrazone formation without oxidation of the hydroxyl group. This was illustrated in the cases of hydroxy-acetaldehyde, acetal, and dihydroxy-acetone. These workers also prepared mesoxaldehyde 1:2-bis-, and tris-(2:4-dinitro-phenyl-hydrazone) and hydroxy-pyruvaldehyde-bis-(2:4-dinitro-phenyl-hydrazone).

Derivatives of Heterocyclic Compounds

Reactions with 5-membered heterocyclic organic compounds have been studied in the cases of 2:3-dihydro-furans and of oxazoles. Boberg *et al.*[13] reported that the reaction of 2:4-dinitro-phenyl-hydrazine with 5-methyl-2:3-diphenyl-2:3-dihydro-furan or 5-methyl-2:3-diphenyl-5-hydroxy-tetrahydro-furan yielded 5-(2:4-dinitro-phenyl-hydrazine)-5-methyl-2:3-diphenyl-tetrahydro-furan. 5-Methyl-2:3-dihydro-furan reacted with ring cleavage to yield the dinitro-phenyl-hydrazone of methyl 3-hydroxy-propyl ketone.

The reaction of oxazoles with 2:4-dinitro-phenyl-hydrazine leads to ring cleavage, yielding the bis-(2:4-dinitro-phenyl-hydrazone) of a substituted glyoxal[14].

An alcoholic solution of glyoxylic acid added at reflux to a solution of 2:4-dinitro-phenyl-hydrazine in 5N sulphuric acid is reported to give the dinitro-phenyl-hydrazone of glyoxal, and hence glyoxal itself[15].

Derivatives of Hydroxy-aromatic Compounds

Hydroxy-aromatic compounds are said to react with excess 2:4-dinitro-phenyl-hydrazine in ethanol $-H_2SO_4$ to yield the bis-(2:4-dinitro-phenyl-hydrazone) of the corresponding quinone, e.g. quinone and catechol give respectively *p*- and *o*-benzoquinone derivatives, while 1:4-dihydroxy-naphthalene yields the mono-2:4-dinitro-phenyl-hydrazone at room temperature and the bis-compound in boiling ethanol[16]. Borsche[17], however, has reported an abnormal reaction with *p*-benzoquinone, the dinitro-hydroxy-azo-benzene being produced.

Triaryl carbinols react with 2:4-dinitro-phenyl-hydrazine to give fair yields of 1-(2:4-dinitro-phenyl)-2-aralkyl-hydrazines.

The reaction is considered to be:

$$(NO_2)_2C_6H_3.NH.NH_2 + Ar_3C.OH \longrightarrow (NO_2)_2.C_6H_3NH.NH.C(Ar)_3$$

where Ar = trityl, phenyl-di-(*p*-tolyl)-methyl, diphenyl-(*p*-tolyl)-methyl, 9-phenyl-9-fluorenyl-1:1-diphenyl-ethyl[18].
A 70% yield of 1-(2:4-dinitro-phenyl)-2-(triphenyl-methyl)-hydrazine has been reported[19].
An interesting exchange reaction between a carbonyl compound and a 2:4-dinitro-phenyl-hydrazone has been described[20]. An excess (x 2) of the carbonyl compound (A) is refluxed for about 24 hours with the dinitro-phenyl-hydrazone (B) and one drop of concentrated hydrochloric acid. Regenerated carbonyl compound (B) was formed in 65-75% yield. The A/B studied were acetophenone/acetone, benzophenone/acetone and benzophenone/acetaldehyde.

Regeneration of Carbonyl Compounds

The regeneration of carbonyl compounds from their dinitro-phenyl-hydrazones may be effected in one of several ways. Thus carbonyl compounds have been regenerated by heating with an excess of laevulinic acid, with the addition of a little hydrochloric acid when $\alpha:\beta$-unsaturated carbonyl compounds are concerned[21]. Generally bis-(2:4-dinitro-phenyl-hydrazones) respond to the treatment: if they do not, regeneration can be effected by adding stronger mineral acid and a solubilising agent, e.g., nitrobenzene. The procedure has been successful in regenerating a keto-steroid from its 2:4-dinitro-phenyl-hydrazone[22]; acetone containing hydrochloric acid has been used for the same type of compound, the acetone dinitro-phenyl-hydrazone being reduced *in situ* with stannous chloride[23].
Robinson[24] reported high yields of ketones from their dinitro-phenyl-hydrazones by heating in a water bath with $\lessapprox 80\%$ formic acid, copper carbonate being added. The 'end-point' was in most cases indicated by the disappearance of colour; the liberated 2:4-dinitro-phenyl-hydrazine is destroyed by oxidation. The reduction of 2:4-dinitro-phenyl-hydrazones has been

studied using lithium aluminium hydride and stannous chloride[25]; the stability of dinitro-phenyl-hydrazones was reported to be greatly diminished by reduction because of the deactivation of the nitro groups[26].

2:4-DINITRO-PHENYL-HYDRAZINE IN QUALITATIVE ANALYSIS

Since its introduction by Brady and Elsmie[2], 2:4-dinitro-phenyl-hydrazine has become a standard laboratory reagent for characterising carbonyl compounds. A solution in 2N hydrochloric acid was originally employed; subsequently Brady[27] suggested an alternative:

1 g of 2:4-dinitro-phenyl-hydrazine is dissolved in 2 ml concentrated sulphuric acid, and 15 ml of ethanol are added; the reagent solution should be freshly prepared. An alcoholic solution containing about 5 milli-mole of carbonyl compound is added to the reagent solution. With aromatic ketones and aldehydes the dinitro-phenyl-hydrazone crystallises at once, and may be collected and washed with cold ethanol. In other cases (some higher aliphatic ketones and aldehydes) it may be necessary to dilute the reaction mixture with 2N sulphuric acid. With fenchone, camphor, acetyl-acetone, and benzoyl-acetone it is recommended that the mixture be allowed to stand overnight.

Allen[28] modified Brady's original method and applied the reagent to a variety of aldehydes, ketones and keto-acids:

A saturated solution of 2:4-dinitro-phenyl-hydrazine is prepared by refluxing 1 g in 100 ml of ethanol. (Partial crystallisation may occur on cooling, but the suspended solid does no harm). To 5 ml of this solution is added 5 ml of ethanol and a few drops of carbonyl compounds, and the whole is carefully heated to boiling. After removal from the flame, 1 to 2 drops of concentrated hydrochloric acid is added, the mixture is boiled for 2 minutes, and water is added drop by drop until cloudiness or crystallisation commences. After cooling, the dinitro-phenyl-hydrazone is filtered, and crystallised from ethanol (low-melting derivatives) or from chloroform or ethyl acetate (high-melting derivatives).

Another method[29] is as follows:

About 0.1 g of 2:4-dinitro-phenyl-hydrazine in 2 ml of ethanol is heated to boiling; concentrated hydrochloric acid is added to the ethanol mixture until the liquid just clears. 0.1 g of the carbonyl compound dissolved in a little ethanol is added, and the whole is heated to boiling and then cooled. The precipitated derivative is filtered and crystallised from hot ethanol without the addition of water.

Solvents

Johnson[30] has suggested a phosphoric acid/ethanol solution of the

reagent; this was found to be stable over a very long period, and the use of the more reactive sulphuric acid is avoided. To prepare 1 litre of approximately 0.25M reagent, 50 g 2:4-dinitro-phenyl-hydrazine were dissolved in 600 ml 85% H_3PO_4 on a steam bath. The solution was diluted with 395 ml 95% ethanol and clarified by filtration through kieselguhr. To a solution of the carbonyl compound in ethanol was added the calculated volume of the reagent solution, after which dilution with water brought about the precipitation of the dinitro-phenyl-hydrazone. Jones *et al.*[31] prepared the 2:4-dinitro-phenyl-hydrazones of eleven dicarbonyl compounds and eight α-substituted carbonyl compounds using 2N hydrochloric acid or Johnson's reagent (ethanolic phosphoric acid) as solvent.

Ethanol is probably the most usual solvent for preparing and crystallising 2:4-dinitro-phenyl-hydrazones, but benzyl alcohol, glacial acetic acid, chloroform, ethyl acetate, xylene, and nitrobenzene have also been employed. In a study of 2:4-dinitro-phenyl-hydrazine as a reagent for carbonyl compounds Campbell[32] successfully used purified industrial methylated spirit as a substitute for pure ethanol in Brady's method. Campbell also drew attention to the variations in reported melting points of dinitro-phenyl-hydrazones in the literature, and recorded fresh melting points of many of these derivatives.

In studies on the visible and ultra-violet light absorption properties in alcoholic and chloroformic solutions, Braude and Jones[33] prepared the dinitro-phenyl-hydrazones of about fifty carbonyl compounds.

The melting points of a number of 2:4-dinitro-phenyl-hydrazones of ketones and aldehydes appear in Appendixes 1 and 2 respectively.

(i) Gravimetric Methods

Several gravimetric determinations of carbonyl compounds have been reported in which the dinitro-phenyl-hydrazone is isolated and weighed. These methods, which tend to give slightly low results because of the solubilities of the derivatives, are generally limited to separations in aqueous media where solubility losses are minimised. The method of gravimetric determination developed by Iddles and Jackson[34] requires only 40×10^{-5} mole of carbonyl compound, so that those aldehydes and ketones normally considered to be insoluble in water can in fact be estimated by this method, which is as follows:

50 ml of a solution (saturated at 0°C) of 2:4-dinitro-phenyl-hydrazine in 2N hydrochloric acid is introduced into a glass-stoppered flask, and to this is added the accurately-weighed sample containing about 40×10^{-5} mole of the aldehyde or ketone. The mixture is allowed to stand in an ice bath for 1 hour, with occasional vigorous shaking when volatile compounds such as acetaldehyde or acetone are being estimated. The precipitate is filtered on a tared Gooch or sintered glass funnel, washed with a little 2N hydrochloric acid and then with water, and finally dried in a vacuum desiccator over sulphuric acid. Most 2:4-dinitro-phenyl-hydrazones can alternatively be dried in a warm oven.

The method has been applied to estimations of formaldehyde, acetaldehyde, propionaldehyde, crotonaldehyde, furfural, salicyaldehyde, anisaldehyde, vanillin, acetone, ethyl methyl ketone, methyl *n*-propyl ketone, methyl vinyl ether, dimethyl acetal, ethyl vinyl ether, and diethyl acetal. Results have been found reproducible to $\pm$ 1%, and the method accurate to $\pm$ 1% in the cases where the dinitro-phenyl-hydrazones are hardly soluble, but slightly low results have been obtained (–2 to –3%) with the more soluble derivatives of acetaldehyde and propionaldehyde.

Subsequently Iddles *et al.*[35] determined the optimum conditions for estimating water-insoluble carbonyl compounds such as benzil. The sample was dissolved in a little ethanol and added to excess 2:4-dinitro-phenyl-hydrazine in 2N hydrochloric acid. After diluting with 50 ml 2N hydrochloric acid and standing at room temperature for a short time, the precipitate was filtered, washed with 2N hydrochloric acid and with water, and dried to constant weight at 105–110°C. In estimating benzil in cloth

samples, recoveries of 97–98% of benzil have been obtained using the method[36].

(ii) Volumetric Methods

Clift and Cook[37] employed a volumetric method for determinations of mesoxalic, aceto-acetic, and oxalo-acetic acids; the 2:4-dinitro-phenyl-hydrazone was prepared and dissolved in excess of standard alkali, the excess NaOH being back-titrated. Espil and Mandillon[38] determined ascorbic acid, ketones and aldehydes in blood and plasma by precipitating the 2:4-dinitro-phenyl-hydrazones and reducing these with standard titanous chloride. The method is as follows:

To 10 ml of the sample 2 ml of 4N sulphuric acid and 15–20 g of anhydrous magnesium sulphate are added. The solid formed is extracted with 80 and then 20 ml of methanol, and 20 ml 2N hydrochloric acid is added to the filtered extracts. Most of the methanol is evaporated under vacuum, and sufficient N/100 iodine is added to the residue to oxidise reducing substances; 10 ml of a saturated solution of 2:4-dinitro-phenyl-hydrazine in dilute hydrochloric acid is added, and the mixture is placed in a refrigerator for 24 hours. The precipitate is collected and washed, first with warm 2N hydrochloric acid and then with cold water. The precipitate, which consists of a mixture of dinitro-phenyl-hydrazones, is dissolved in N/2 sodium carbonate, from which solution the 2:4-dinitro-phenyl-hydrazone of ascorbic acid is precipitated by passing in carbon dioxide, leaving the other dinitro-phenyl-hydrazones in solution. The precipitate is collected and dissolved in methanol with the aid of hydrochloric acid, and the nitro-groups are reduced with excess of standard $TiCl_3$ at 50 C under an atmosphere of CO_2. Excess of $TiCl_3$ is titrated with standard ferric ammonium sulphate. The other hydrazones are determined in a similar manner. (1 mol 2:4-dinitro-phenyl-hydrazine requires 12 equivalents of $TiCl_3$.)

A similar method was recommended by Schoniger and Lieb[39] for carbonyl groups in general, the excess $TiCl_3$ being titrated with standard ferric alum to a thiocyanate end-point.

Zobov and his co-workers[40,41] have studied amperometric titrations of aldehydes and ketones with solutions of 2:4-dinitro-phenyl-hydrazine, and have recommended the use of ultrasonic waves to coagulate the precipitated dinitro-phenyl-hydrazones.

(iii) Colorimetric Methods

The addition of sodium or potassium hydroxide to an alcoholic solution of a 2:4-dinitro-phenyl-hydrazone produces an intense wine-red colour. This reaction was employed by Lappin and Clark[42] as the basis of a colorimetric method for determining traces of carbonyl compounds, and was applied to aldehydes and ketones in water, organic solvents, and in organic reaction products. The method was found to be most useful in the range 10^{-4} to 10^{-6}M of carbonyl.

A spectrophotometric procedure, based on Lappin and Clark's method, was recommended by Mendelowitz and Riley[43] for estimating ketonic groups in long-chain fatty acids, particularly in licanic acid.

Toren and Heinrich[44] failed to obtain reproducible results with Lappin and Clark's method under the conditions specified, and recommended reaction in a two-phase system, ethanol/water/phosphoric acid and *iso*-octane. The 2:4-dinitro-phenyl-hydrazone was preferentially extracted by the *iso*-octane, excess reagent remaining in the aqueous phase; absorbance was measured at 340 mμ. This work was extended by Lohman[45], and applied to estimations of carbonyl compounds in the range 3 to 300 p.p.m. of carbonyl oxygen, the dinitro-phenyl-hydrazone being extracted from excess 2:4-dinitro-phenyl-hydrazine with hexane; the absorbance was measured at 340 mμ.

Jones *et al.*[46,47] made spectrophotometric studies of many 2:4-dinitro-phenyl-hydrazones, and examined the ultra-violet, visible and infra-red spectra of forty of these derivatives[48].

Aldehydes and Benzophenone

Perez[49] used the following method for estimating aldehydes and benzophenone: 10–100 μg sample is dissolved in 1 ml acetic acid, and 5 ml of a 0.1% solution of 2:4-dinitro-phenyl-hydrazine in acetic acid containing 0.5% of concentrated hydrochloric acid is added. The mixture is kept in the dark for 1 hour at room temperature (or 15 minutes at 100°C for benzophenone, camphor and sugars). The absorbance of the stable red solution is measured at 412.4 mμ (432.5 mμ for benzophenone, camphor and sugars).

Secondary Alcohols

Critchfield and Hutchinson[50] described a determination of secondary alcohols in the presence of primary alcohols. The method depends on the oxidation of secondary alcohol to ketone,

using acid potassium dichromate, the amount of ketone formed being estimated colorimetrically as the dinitro-phenyl-hydrazone.

Alkaloids

Alkaloids have been determined by a method described by Sakuria[51], in which oxidation with ammonium ceric nitrate and treatment with 2:4-dinitro-phenyl-hydrazine give an orange-red colour with codeine and morphine; thebaine, narcotine and papaverine give yellow compounds. 1.5 μg/0.5 ml can be determined.

Pyruvic acid

During studies of pyruvic acid metabolism, in normal and in Vitamin B deficient states, Lu[52] developed a rapid, specific and sensitive test for estimating blood pyruvate. The pyruvic acid in a trichloro-acetic acid extract of blood was converted to the 2:4-dinitro-phenyl-hydrazone, which was extracted with ethyl acetate. The coloured compound was extracted from the ethyl acetate with 10% sodium carbonate solution, treated with sodium hydroxide to produce a stable red colour, and estimated colorimetrically using a previously prepared calibration curve. The method was claimed to estimate 2 μg pyruvic acid in 10 ml; other keto-acids were reported to cause but little interference. As a significant decrease in pyruvic acid content was observed when a blood sample stood for even 1 minute at room temperature Bueding and Wortis[53] modified Lu's method by using a stabilising agent. Adding 0.2% of sodium iodo-acetate was claimed to inhibit completely the loss of pyruvic acid. The same workers determined pyruvic acid in blood and in cerebro-spinal fluid, from samples taken simultaneously[54].

Friedmann and Haugen[55] also reported a modification of Lu's method, and used it for determining pyruvic acid and total keto acids in blood and in urine.

Hexosamines

Popowicz[56] has described an interesting method for determining hexosamines in biological materials, based on deamination with nitrous acid and reaction of the resulting 2:5-anhydro-hexoses with 2:4-dinitro-phenyl-hydrazine. The coloured compounds were estimated photometrically in the range 10–100 μg.

Critchfield's method for aldehydes and ketones

The use of pyridine as a stabiliser in 2:4-dinitro-phenyl-

hydrazine determinations of acetaldehyde was recommended by Böhme and Winkler[57], who subsequently extended the method to estimations of small quantities of acetone, especially in blood and in urine[58]. The same workers reported a photometric procedure for determining benzaldehyde and vanillin in foodstuffs, based on the colour reaction of the dinitro-phenyl-hydrazone in alkaline solution[59]. More recently Critchfield[60] has employed pyridine as stabiliser in the following method for determining low concentrations of aldehydes and ketones:

Reagents

Carbonyl-free methanol

500 ml reagent grade methanol are refluxed for 2 hours with 5 g of 2:4-dinitro-phenyl-hydrazine and a few drops of concentrated hydrochloric acid. After distillation through a short Vigreux column the methanol is kept in a tightly stoppered bottle, where it remains suitable for use for several months.

2:4-Dinitro-phenyl-hydrazine reagent

50 mg of the solid is dissolved in 25 ml of carbonyl-free methanol, 2 ml of concentrated hydrochloric acid is added, and the whole diluted to 50 ml with distilled water. (Stable for about 2 weeks.)

Pyridine stabiliser

80 volumes of reagent grade pyridine are mixed with 20 volumes of distilled water.

Potassium hydroxide solution

100 g of potassium hydroxide is dissolved in a little water and the volume is made up to 300 ml with carbonyl-free methanol.

Method

From a total volume of 100 ml, consisting of a solution of not more than 40μmole of carbonyl compound in carbonyl-free methanol, 2 ml is pipetted into one of two 25 ml stoppered graduated cylinders; 2 ml of carbonyl-free methanol is pipetted into the other cylinder to serve as a blank. 2 ml of the 2:4-dinitro-phenyl-hydrazine solution is added to each cylinder, and after mixing the contents are allowed to stand for 30 minutes. 10 ml pyridine stabiliser is added to each cylinder, followed by 2 ml potassium hydroxide solution. After mixing, the absorbance (optical density) is measured at 480 mμ in 1 cm cells, in 10$\pm$ 1 minutes after adding the potassium hydroxide solution. The carbonyl concentration is obtained by reference to a calibration curve prepared from standards.

Enzyme Assays

The formation and measurement of coloured dinitro-phenyl-hydrazone solutions are the basis of a number of enzyme assay methods[61]. Determinations of lactic dehydrogenase (LDH) are based on the catalysis of pyruvic acid to lactic acid in the presence of 2:4-dinitro-phenyl-hydrazine, and the formation of a coloured dinitro-phenyl-hydrazone of the residual pyruvic acid from the pyruvate substrate[62].

Glutamic-oxaloacetic transaminase (SGO-T) and glutamic-pyruvic transaminase (SGP-T)[63] are also assayed by colorimetric methods in which 2:4-dinitro-phenyl-hydrazine is employed. In the case of SGO-T the quantitative determination of the enzyme is based on the transamination of L-aspartic and oxoglutaric acids to oxaloacetic and glutamic acids respectively. The oxaloacetic acid is decomposed by aniline citrate to pyruvic acid which is converted to the dinitro-phenyl-hydrazone. Similarly the SGP-T assay is based on the transamination of L-alanine and α-oxo-glutaric acid to pyruvic and glutamic acids, the pyruvic acid being estimated colorimetrically as the 2:4-dinitro-phenyl-hydrazone.

CHROMATOGRAPHIC SEPARATIONS OF 2:4-DINITRO-PHENYL-HYDRAZONES

Column Chromatography

In 1935 Strain[3] described the separation of the 2:4-dinitro-phenyl-hydrazine derivatives of β-ionone and camphor, and of geronic acid and laevulinic acid on a talc column. In the same year Lucas *et al.*[64] used an alumina column for separating the 2:4-dinitro-phenyl-hydrazones of acetaldehyde and propionaldehyde; alumina was also used for separating 2-cyclo-butyl-cyclobutenones via their 2:4-dinitro-phenyl-hydrazones[65], and for separating certain androgens by a similar technique[66]. Datta *et al.*[67] developed a quantitative method for estimating keto-acids in urine and in other body fluids. The mixture of 2:4-dinitro-phenyl-hydrazones in ethyl acetate was separated on an alumina column, followed by elution with a series of solvents, 15 ml fractions being taken for absorption measurements of the yellow colour in a 1 cm cell, using Chance OB1 blue light filter. Quantities of keto-acids of the order of 1 mg were estimated with an error of $< 5\%$.

White[68] separated many aliphatic dinitro-phenyl-hydrazones on a bentonite column with diethyl ether and/or hexane. Of 22 pairs examined, representing 12 aliphatic aldehydes and ketones, 18 pairs were separated satisfactorily; among these were mixtures of acetone and ethyl methyl ketone; acetaldehyde and butyraldehyde; *n*-butyraldehyde and *iso*-butyraldehyde.

Recently Matheson[69] has isolated amino acids from mixtures by separating the 2:4-dinitro-phenyl-hydrazine derivatives by column chromatography.

A method developed by Pool and Klose[70], applicable to estimations of monocarbonyl compounds in rancid foods, is as follows:

A chromatographic column 7 mm x 110 mm has 3 cm of alumina placed in it; 10 ml of a solution of 2:4-dinitro-phenyl-hydrazine (500 mg of the solid dissolved in 1 litre of benzene with warming) is introduced into the column by pipette, and alumina is added to a depth of 10 to 11 cm. 5 ml of benzene is added, followed by the sample containing 0.05–0.5 μmole –CHO in solution in 3–4 ml of benzene. The solution issuing from the column is collected in a 25 ml measure. Benzene is added to the column until the volume collected is 19 ml. This is diluted to 25 ml with alcoholic potassium hydroxide (60 g KOH/litre of 99% aldehyde-free ethanol), and the absorbance is measured at once at 435 mμ. A blank is run at the same time. Excess reagent and

dinitro-phenyl-hydrazones of dicarbonyl compounds are held up by the column. The authors of the method prepared calibration curves for 6 aldehydes, showing the relation between absorbance and concentration (mole/litre).

In a method for determining biologically active pyrethrins in insecticides, Camoni and Crudelli[71] extracted the 2:4-dinitro-phenyl-hydrazones with hexane; the derivatives of pyrethrins I and II were separated on an alumina column and after elution were determined spectrophotometrically.

More recently Head[72] has developed a rapid method for the analysis of pyrethrum extract, etc., in which pyrethrins are converted to their 2:4-dinitro-phenyl-hydrazones and, without chromatography, the optical density is measured at 377 mμ. A value for the molecular extinction coefficient $\varepsilon = 2.8 \times 10^4$ is used for calculating pyrethrin concentrations.

Schwartz *et al.*[73] employed a magnesia/'Celite' column for separating mono-carbonyl compounds in the form of their 2:4-dinitro-phenyl-hydrazones. Four classes of compound, *viz.* methyl ketones, saturated aldehydes, 2-enals and 2:4-dienals, were reported to separate in that order on elution with chloroform in hexane. The separation of the classes could be followed visually, the colours being respectively grey, tan, rust-red and lavender.

A similar method has been reported for the direct quantitative isolation of monocarbonyl from fats and oils, cheese extracts and whole milk powders[74]. A modified Pool and Klose procedure has been employed for determining free mono-carbonyl compounds in edible fats[75]; Lea and Jackson[76] have described a colorimetric method for activating the volatile or 'free' carbonyl in fats, after chromatography in an alumina column.

The need for carbonyl-free solvents in chromatographic analyses of carbonyl compounds has resulted in the development of several methods of purification. Begemann and de Jong[77] used a 'Celite' column as a carrier for 2:4-dinitro-phenyl-hydrazine; solvents containing traces of carbonyl impurities are passed through the column, –CO being almost quantitatively bound.

Schwartz and Parks[78] have used a similar method, claimed to be an improvement on that of Begemann and de Jong, for preparing carbonyl-free solvents for use in the micro-analysis of carbonyl compounds.

Corbin *et al.*[79] used liquid/liquid partition chromatography in a 'Celite' column, with methyl cyanide or 2-chloro-ethanol as the stationary phase, and demonstrated the separation of 2:4-dinitro-phenyl-hydrazones or bis-(2:4-dinitro-phenyl-hydrazones) of saturated aldehydes, methyl ketones, 2-enals and 2:4-dienals. A three-column system was subsequently developed, and with methyl cyanide/water mixtures as stationary phases on 'Celite' columns 2:4-dinitro-phenyl-hydrazones of highly polar carbonyl compounds from oxidised whole milk powder were separated and estimated[80].

Paper Chromatography

Cavallini *et al.*[81] characterised keto-acids of biological interest using partition chromatography, the 2:4-dinitro-phenyl-hydrazones being separated on filter paper, with butanol : ethanol : water or butanol : 3% ammonia : water as solvents. Well-separated spots were obtained for the 2:4-dinitro-phenyl-hydrazones of several α-keto-acids, *viz.* α-keto-glutaric, oxaloacetic, glyoxylic, pyruvic, aceto-acetic, α-keto-γ-methio-butyric, α-keto-butyric, *p*-hydroxy-phenyl-pyruvic, and phenyl-pyruvic acids. These workers adapted the method to determinations of the keto-acid content of human blood and urine[82].
Subsequently Altmann *et al.*[83] studied in detail the paper chromatography of keto-acids, and found that it was possible to separate the 2:4-dinitro-phenyl-hydrazones as compact spots, and to obtain accurate and reproducible Rf values. The paper used was Whatman No. 2, washed with 0.1M glycine-NaOH buffer, pH 8.2–8.4, and the most satisfactory solvent used was *tert.*-amyl alcohol: ethanol : water = 50 : 10 : 40. Under these conditions the recorded Rf values were:

Keto-acid	Rf value
Oxaloacetic	0.08
α-Keto-glutaric	0.16
Pyruvic	0.36
Aceto-acetic	0.50
p-Hydroxy-phenyl-pyruvic	0.70
Phenyl-pyruvic	0.78
o-Hydroxy-phenyl-pyruvic	0.81

Seligman and Shapiro[84] also made a study of β-keto-acids in blood and in urine using paper chromatography, and developed a method based on that of Cavallini *et al.*, claimed to be accurate and specific for individual keto-acids.

The method depends on the preparation of 2:4-dinitro-phenyl-hydrazones of the keto-acids present in the sample, and extraction of these derivatives, followed by paper chromatography. After drying the chromatogram the keto-acid dinitro-phenyl-hydrazone areas are traced out with pencil under ultra-violet light; the keto-acids are identified by Rf measurements or by markers of pure keto-acid 2:4-dinitro-phenyl-hydrazones. The marked areas are cut out, sliced into small pieces, and shaken with N sodium hydroxide to elute the dinitro-phenyl-hydrazones. After filtering, the red colour of the filtrate is determined using a 455 filter.

Subsequently Isherwood and Cruikshank[85] pointed out that α-keto-acids can give rise to two stereo-isomeric 2:4-dinitro-phenyl-hydrazones which do not give identical colour reactions in dilute sodium hydroxide. To eliminate any uncertainty, these workers modified the method previously reported by Cavallini *et al.* After separation of the dinitro-phenyl-hydrazones by paper chromatography it was shown that the isomers of pyruvic, oxaloacetic, and α-keto-glutaric acids were probably cis-trans geometrical isomers. Quantitative measurements were made by determining the extinction of eluted 2:4-dinitro-phenyl-hydrazones at 365 and 404 mμ (mercury lines).

In the case of pyruvic acid the sum of the contributions of both 2:4-dinitro-phenyl-hydrazones was taken; with oxaloacetic acid, both forms were estimated together; α-keto-glutaric acid gave only one form under the standard conditions described.

In these studies *tert.*-amyl alcohol : *n*-propanol : conc. ammonia = 65:5:30 was used with unbuffered paper, or *tert.*-amyl alcohol : ethanol : water = 50:10:40 on paper dipped in 0.05M phosphate buffer (pH = 8.0) and air dried.

Simple aliphatic aldehydes and ketones have been separated quantitatively by paper chromatography *via* the 2:4-dinitro-phenyl-hydrazones in a methanol/ligroin mixture, with 10% acetic acid in ligroin as the moving phase[86]. Higher aldehydes and ketones could be identified by this method, but quantitative separations were not possible because of overlap.

Separation and identification of 2:4-dinitro-phenyl-hydrazones by paper chromatography have also been carried out by Rice *et al.*[87]. Among the solvent systems employed were 5% diethyl ether in petroleum spirit 65–110°C; aqueous acetone : petroleum spirit 20–40°C = 98:2; and tetrahydrofuran : petroleum spirit 65–110°C = 30:70. After chromatography the paper was sprayed with 10% aqueous potassium hydroxide, and the coloured areas (from the reaction of the dinitro-phenyl-hydrazones with KOH) were outlined in pencil. The method was reported to be simple and rapid, and to require only μg quantities of dinitro-phenyl-hydrazones.

Following an intensive study of the chromatographic properties of some ketones, aldehydes, and keto-acids in acid systems, Bush and Hockaday[88] reported that running times in paper chromatographic separations were less than in alkaline systems. Acid systems were found to be very convenient in determinations of α-keto-acids. Quantitative measurements were carried out either by a direct scanning of the paper strips or by measuring the absorbance of the eluted zones.

Thin-layer Chromatography (TLC)

A number of workers have employed the TLC technique in separations of 2:4-dinitro-phenyl-hydrazones. Rosmus and Deyl[89] used centrifugal paper chromatography and TLC. In an investigation of volatile carbonyl compounds from foods Dhont and Rooy[90] used a TLC method for separating the 2:4-dinitro-phenyl-hydrazones, and recorded Rf values for the derivatives of several natural compounds in two solvent systems. Auviken and Favorskaya[91] have described the micro-preparation of 2:4-dinitro-phenyl-hydrazones and their separation by TLC, which has also been used for separating aliphatic 2:4-dinitro-phenyl-hydrazones[92].

2:4-DINITRO-PHENYL-HYDRAZINE IN THE STEROID FIELD

The properties which have made 2:4-dinitro-phenyl-hydrazine such a useful reagent for characterising aliphatic and aromatic carbonyl groups, have been applied with advantage in several aspects of steroid work. In a study of the oxidation of phytosterols with the Oppenauer reagent, Jones *et al.*[93] prepared 2:4-dinitro-phenyl-hydrazones of the resulting ketones, *viz.* cholestenone, fucostadienone, stigmastadienone and sitostenone.

Some Melting Points and U/V Spectra

Djerassi and Ryan[94] determined and tabulated the ultra-violet absorption spectra of a number of steroidal and other dinitro-phenyl-hydrazones; Reich *et al.*[95] employed a method for preparing and isolating quantitatively a number of oxo-steroids, in which excess 2:4-dinitro-phenyl-hydrazine was added, the excess being determined as the acetone or pyruvic acid dinitro-phenyl-hydrazone; by this means the quantity of 2:4-dinitro-phenyl-hydrazine associated with a given keto-steroid was determined. The melting points and ultra-violet maxima of several new dinitro-phenyl-hydrazones were reported; all were purified by chromatography on alumina.

Under the conditions used, *viz.* precipitation of 2:4-dinitro-phenyl-hydrazones from alcoholic solutions in the presence of small amounts of mineral acid, steroids containing keto groups in the 3-, 6-, 7-, 12-, 16-, 17-, and 20- positions reacted rapidly with 2:4-dinitro-phenyl-hydrazine (the 11-keto group is known to be unreactive towards carbonyl reagents).

Gravimetric Determination of Progesterone

Klein *et al.*[96] who described a gravimetric method in which crystalline progesterone was identified by weighing the 2:4-dinitro-phenyl-hydrazone, showed that 2 mol. 2:4-dinitro-phenyl-hydrazine are involved with 1 mol. of the steroid and postulated a monopyrazoline-monohydrazone structure isomeric with the expected progesterone bis-(2:4-dinitro-phenyl-hydrazone). Djerassi[97], however, has shown that the compound is the bis-(2:4-dinitro-phenyl-hydrazone) itself. Madigan *et al.*[98] reported that Klein's method was not applicable to testosterone.

Colorimetric Determinations

Gormall and MacDonald[99] estimated steroid hormones colorimetrically in extracts of urine, plasma and tissue, using

BDH 'AnalaR' 2:4-dinitro-phenyl-hydrazine which was found to be perfectly satisfactory for steroid work without further purification.

General Preparations of Steroid Derivatives

Reich and Samuels[100] studied the preparation of oxo-steroid 2:4-dinitro-phenyl-hydrazones, and reported that none of the steroids investigated reacted quantitatively when Gormall and MacDonald's method was applied, the amount of 2:4-dinitro-phenyl-hydrazine used for a single keto group ranging from 0.45 to 0.85 mol.

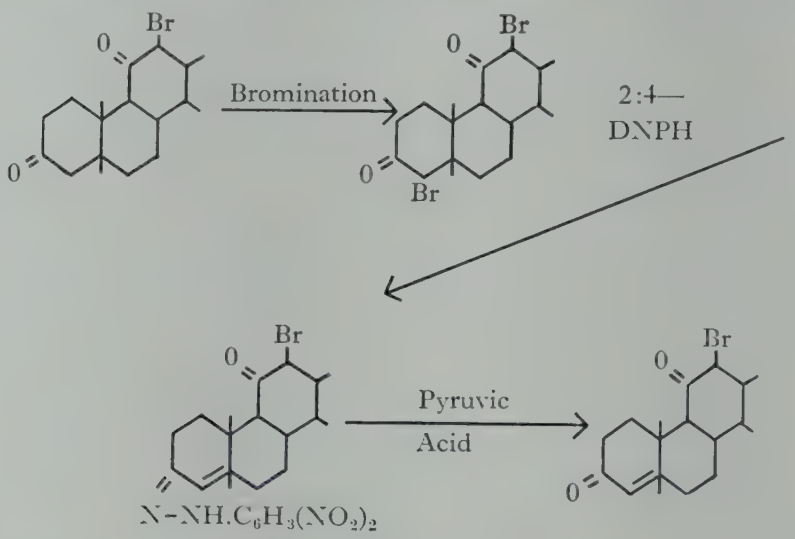
Two steroids with a ketol side chain, 21-hydroxy and 21-acetoxy-pregnenolone, gave, according to conditions, either the normal 2:4-dinitro-phenyl-hydrazone or pregna-5-ene-3β-ol-20-one-21-al bis-(dinitro-phenyl-hydrazone). The latter could be obtained from pregna-5-ene-3β:17α:21-triol-20-one when the reaction was carried out at 60°C, but at room temperature a dehydration took place and the dinitro-phenyl-hydrazone of pregna-5:16-diene-3β-21-diol-20-one was formed.

Reactions of Adrenocortical Hormones

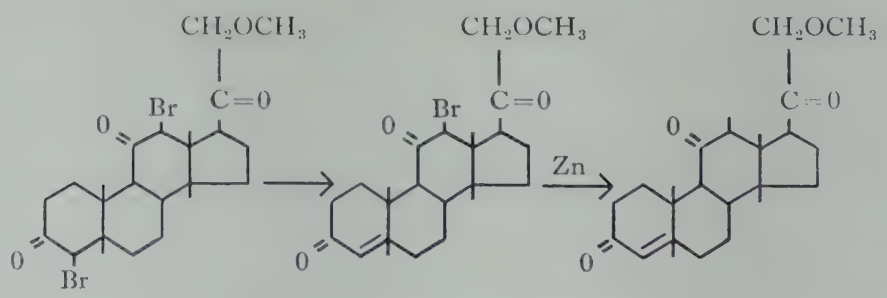
These workers subsequently[101] studied the reactions of adrenocortical hormones with 2:4-dinitro-phenyl-hydrazine, and prepared the bis-(2:4-dinitro-phenyl-hydrazone) of deoxycortisone, pregn-4-en-17α:21-diol-3:20-dione, cortisone and cortisol. The 21-monoacetates of the four cortico-steroids were converted to the 3-mono-2:4-dinitro-phenyl-hydrazones; these were reported to be suitable derivatives for spectrophotometric estimations.

Introduction of Double Bonds

A new method for introducing a double bond between the carbon atoms in the 4:5 positions in 3-oxo-steroids was reported by Mattox and Kendall[102]. For example, methyl-3:11-diketo-12-bromo-cholanate was brominated to yield methyl-3:11-diketo-4:12-dibromo-cholanate, which was converted to the 3-(2:4-dinitro-phenyl-hydrazone), hydrobromic acid being evolved quantitatively without the use of sodium acetate. Removal of the 2:4-dinitro-phenyl-hydrazone group from the 3-position with pyruvic acid gave the 3-oxo-Δ^4-steroid.



Mattox and Kendall[103] also employed the technique for introducing a double bond into certain adrenal cortical hormones, *e.g.*, 3:11:20-triketo-4:12α-dibromo-21-acetoxy-pregnane gave a good yield of the pregna-4-ene:



Under the conditions used the carbonyl group at C20 does not form a dinitro-phenyl-hydrazone. When treated with 2:4-dinitro-phenyl-hydrazine in acetic acid 3:11:20-triketo-2-bromo-17α-hydroxy-21-acetoxy-pregnane yielded a mixture of the Δ^1-hydrazone and 2-acetoxy-hydrazone; the latter was converted to the Δ^1-hydrazone by heating in a solution of acetic acid containing HBr.

Regeneration of Steroids from their Derivatives

The scope and mechanism of the Mattox-Kendall reaction was studied by Djerassi[97], who also determined the ultra-violet absorption spectra of a number of steroidal 2:4-dinitro-phenyl-hydrazones. The high yields of Δ^1- and Δ^4-3-ketones were confirmed but it was pointed out that in preparations of, for example, the 4:6-dien- and 1:4-dien-oxo steroids the regeneration of the unsaturated carbonyl compounds from the 2:4-dinitro-phenyl-hydrazones gave low yields, thus imposing limitations on the Mattox-Kendall reaction. The procedure reported by Demaecker and Martin[23] was claimed to overcome this difficulty. The dinitro-phenyl-hydrazone was refluxed with acetone and HC1 and the solution treated with $SnCl_2$ in HCl. After recovery of acetone the residue was taken up in benzene, washed in HC1 until no more coloured material was removed, and the benzene distilled off.

This procedure gave a 95% yield of cholest-4-en-3-one, 84% of cholest-1-en-3-one, 83% of cholesta-1:4-dien-3-one, 88% of cholesta-4:6-dien-3-one and 67% of methyl-3-keto-etiochola-1:4-dienate. The method was applied by Beereboom and Djerassi[104] to the preparation of 2-chloro-cholest-1-en-3-one from 2:2-dichloro-cholestanone.

The recovery of carbonyl compounds from their 2:4-dinitro-phenyl-hydrazones has been effected in several different ways (see page 7). Exchange reactions with pyruvic or laevulic acid in the presence of mineral acids have been successful in some instances, though the conditions are somewhat severe for sensitive structures; this limitation also applies to the use of acetone, or reduction of the nitro-groups with stannous chloride. Elks and Oughton[105] have recommended the use of chromous chloride for reducing the nitro-groups in recovering 3-oxo-steroids from their dinitro-phenyl-hydrazones. A two-phase system was employed, the regenerated steroid being protected by the water-immiscible phase from hydrolysis by the aqueous acid, the whole regeneration being carried out in an inert atmosphere.

SOME MISCELLANEOUS APPLICATIONS

In several manufacturing processes for acrylonitrile, the small quantities of methyl vinyl ketone also formed are claimed to be removed by treatment with 2:4-dinitro-phenyl-hydrazine[106].
The 2:4-dinitro-phenyl-hydrazides of acetic, formic, propionic and *iso*-butyric acids are reported to be effective herbicides when applied to soils before seedling emergence[107].
Addition compounds, obtained by treating an organic aromatic or heterocyclic N-containing base with 2:4-dinitro-phenyl-hydrazine in hydrochloric acid or sulphuric acid solution, consist of 1 mol. base:1 mol. 2:4-dinitro-phenyl-hydrazine:2 equivalents of acid[108]. They are readily decomposed by water, and have been recommended for preparative or analytical purposes[109].
Barakat *et al.*[110] have described the preparation of a new indicator. By condensing sodium 1:2-naphthaquinone-4-sulphonate with 2:4-dinitro-phenyl-hydrazine an *o*-hydroxy-azo compound is formed, a solution of which is rose red at pH 8.4 and violet at 9.2. Among other advantages claimed for the indicator are: it is easily prepared in crystalline form; it is soluble in water, ethanol and ethanol/diethyl ether mixtures; it changes colour over a narrow pH range; it is very sensitive, even with N/1000 solutions; it is suitable for acid vs. base or base vs. acid titrations.

Laboratory Packages and Specifications
BDH supplies pure and 'AnalaR' grades of 2:4-dinitro-phenyl-hydrazine in 25 g, 100 g, and 250 g laboratory packages.

Specifications for the two grades are:

2:4-DINITRO-PHENYL-HYDRAZINE

Melting point	197–202°C
Sulphated ash	Not more than 0.05%

2:4-DINITRO-PHENYL-HYDRAZINE 'ANALAR'

Melting point	196–199°C
Sulphated ash	Not more than 0.02%

Both are also available in bulk quantities

APPENDIX I

MELTING POINTS OF 2:4-DINITRO-PHENYL-HYDRAZONES OF ALDEHYDES

Aldehyde	M.p. °C	Aldehyde	M.p. °C
Acetaldehyde	168 (167)	*o*-Hydroxy-benzaldehyde	252
Acrolein	165	*m*-Hydroxy-benzaldehyde	260 d.
m-Amino-benzaldehyde	270–1	*p*-Hydroxy-benzaldehyde	280 d.
α-*n*-Amyl cinnamic aldehyde	164	Lauric aldehyde *see* *n*-Duodecaldehyde	
Anisaldehyde	253.4 d.	*o*-Methoxy-benzaldehyde	253
Benzaldehyde	237	*m*-Methoxy-benzaldehyde	218–9
iso-Butyraldehyde	182	*p*-Methoxy-benzaldehyde *see* Anisaldehyde	
n-Butyraldehyde	126 (122)	α-Methyl-acrolein	206
n-Caproaldehyde	104	α-Methyl-β-ethyl-acrolein	159
o-Chloro-benzaldehyde	206–7	α-Naphthaldehyde	270
p-Chloro-benzaldehyde	264–5	*o*-Nitrobenzaldehyde	250 d.
Cinnamaldehyde	255 d.	*m*-Nitrobenzaldehyde	292–3 d.
Citral a	116 (108–10)	*p*-Nitrobenzaldehyde	320
Citral b	96	*n*-Nonyl aldehyde	96 (100)
Citronellal	77 (78)	*n*-Octaldehyde	106
Crotonaldehyde	190	Phenyl-acetaldehyde	121
Cuminaldehyde	243 (241)	Piperonyl aldehyde	266 d.
n-Decylaldehyde	104	Propionaldehyde	154 (155)
2:4-Dihydroxy-benzaldehyde	286 d.	Salicylaldehyde	252 d.
3:4-Dihydroxy-benzaldehyde	275 d.	Tiglaldehyde	215
3:4-Dimethoxy-benzaldehyde	264	*o*-Toluic aldehyde	194
2:4-Dinitro-benzaldehyde	264	*m*-Toluic aldehyde	194
p-Dimethyl-amino-benzaldehyde	237 (233–4)	*p*-Toluic aldehyde	234
n-Duodecylaldehyde	106	Trimethyl-acetaldehyde	210
Formaldehyde	166 (168)	2:4:6-Trinitrobenzaldehyde	208
Furfuraldehyde	202	*n*-Undecylaldehyde	104
Glyoxal	328	*n*-Valeraldehyde	98 (106–7)
n-Heptaldehyde	108 (106)	*iso*-Valeraldehyde	123
Hexanal	104	Vanillin	271 d.
Hydrocinnamaldehyde	149		

APPENDIX II

MELTING POINTS OF 2:4-DINITRO-PHENYL-HYDRAZONES OF KETONES

Ketone	M.p. °C	Ketone	M.p. °C
Acetoacetic acid	125	Cyclo-octanone	163
Acetoacetic ester	93.4	Cyclo-pentadecanone	105
Acetol—*see* Hydroxy-acetone		Cyclopentanone	146–7 (142)
Acetone	126 (128)	Diacetone alcohol	203
Acetonyl-acetone	257	Diacetyl ketone	315
Acetophenone	250 (237)	Dibenzal-acetone	180
Acetyl-acetone	209	Dibenzyl ketone	100
2-Acetyl-naphthalene	262	Di-*iso*-butyl ketone	92
Allyl-acetone	104	Dicinnamylidine acetone	208
m-Amino-acetophenone	265–6	Diethyl ketone	156
p-Amino-acetophenone	258–9	Di-*iso*-propyl ketone	98
Benzal-acetone	227 (223)	Di-*n*-propyl ketone	75
Benzal-acetophenone	224 d. (208)	Di-*p*-tolyl ketone	229
Benzil	189 (185)	Ethyl aceto-acetate	96
Benzoin	245 (234)	Ethyl *iso*-butyl ketone	75
Benzophenone	238–9	Ethyl levulate	101
Benzoyl aceto-acetic ester	222–3	Ethyl methyl ketone	110–11 (115)
Benzoyl-acetone	151	Ethyl oxomalonate	128
p-Benzoyl-diphenyl	214	Ethyl *iso*-propyl ketone	112
2-Benzoyl-naphthalene	257–8	Ethyl *n*-propyl ketone	130
Benzyl methyl ketone	156	Fenchone	140
n-Butyroin	99	Fluorenone	284
n-Butyrophenone	190	Furil	215
Carvone	189	Furoin	216–7
D-Carvone	190	α-Hydrindone	258
L-Carvone	193	Hydroxy-acetone	129
D-Camphor	177	*p*-Hydroxy-acetophenone	261
Chalcone	248	α-Indanone	258
p-Chloro-acetophenone	235–6	α-Ionone	150
Chrysoquinone	308–9 d.	Levulinic acid	206
Cinnamal-acetone	222	Menthone	145
Cinnamalydine acetophenone	218–9 d.		
Cycloheptanone	148		
Cyclohexanone	162 (160)		

Ketone	M.p. °C	Ketone	M.p. °C
Mesityl oxide	203 (200)	Pseudoionone	143
p-Methoxy-acetophenone	220	Pulegone	147
m-Methyl-acetophenone	207	Pyruvic acid	218
o-Methyl-acetophenone	159	β-Thujone	116–7
p-Methyl-acetophenone	256		
Methyl *iso*-amyl ketone	95		
Methyl *n*-amyl ketone	89		
Methyl benzoyl formate	171		
Methyl *iso*-butyl ketone	95		
Methyl *n*-butyl ketone	106		
2-Methyl-cyclohexanone	137		
3-Methyl-cyclohexanone	155		
4-Methyl-cyclohexanone	134		
Methyl cyclohexyl ketone	140		
Methyl heptenone	81		
Methyl *iso*-hexyl ketone	77		
Methyl *n*-hexyl ketone	58		
Methyl β-naphthyl ketone	262		
Methyl *n*-nonyl ketone	63		
Methyl *iso*-propyl ketone	117		
Methyl *n*-propyl ketone	143 (141)		
Methyl *p*-tolyl ketone	248 (257–8)		
Methyl *n*-undecyl ketone	69		
m-Nitro-acetophenone	227–8		
p-Nitro-acetophenone	257–8		
Phenanthraquinone	312–3 d.		
Phenyl benzyl ketone	204		
Phenyl *p*-tolyl ketone	200		
Phorone	112		
iso-Phorone	130		
Pinacolone	125		
Piperitone	119		
Propiophenone	190		

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